

Histological appearance of ipsilateral submental lymph nodes at 24 h after injection of ferritin to hamster cheek pouch (A) and at 2 h after inoculation of hamster lip (B) (Gomori's iron stain, $\times 450$). Black granules represent iron deposits (arrows).

In any case, this situation of a reduced and delayed lymphatic drainage from the hamster cheek pouch affords foreign grafts in this site a decisive advantage for prolonged survival, particularly also because of the availability of a rich and plastic vascular supply⁶.

Zusammenfassung. Zur Untersuchung des Lymphabflusses beim Hamster wurde Ferritin in die Backentaschen und Lippen der Tiere injiziert. 2 h nach der Injektion in die Lippe fanden sich grosse Mengen des Ferritins im ipsilateralen submentalen Lymphknoten, während sich nach 24 h und nach Injektion in die Backentasche nur wenig Ferritin fand, was als Zeichen

einer immunologischen Bevorzugung der Hamster-Backentasche angesehen wird.

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Coagulation of Hemolymph of the Larval Honey Bee (*Apis mellifera* L.)

Previous investigations in the field of invertebrate blood, or hemolymph, coagulation have consisted primarily of morphological description rather than identification of the chemical substances involved in the clotting process. For example, GREGOIRE¹⁻³ studied patterns of blood coagulation in various insects by using phase-contrast microscopy. The purpose of this investigation was to determine whether the hemolymph of the honey bee, *Apis mellifera* L., contains clotting factors similar to those found in human blood and why the hemolymph does not coagulate.

Hemolymph was obtained by gently puncturing larvae of various ages with a sterile hypodermic needle and drawing the fluid which exuded from the wound into a capillary pipette. The hemolymph was pooled in shell vials and stored at -20°C . (Blood of pupae and adults was not used because of the difficulty of obtaining sufficient quantities.) This pooled larval honey bee hemolymph was then tested for the presence of the blood coagulants that exist in human blood.

Normal plasma and serum obtained from human volunteers were used as standards. Insect hemolymph was treated in exactly the same manner as human blood. The

following determinations for coagulants were made in triplicate on each sample using the methods of EICHELBERGER⁴:

1. One-stage prothrombin time.
2. Partial thromboplastin time.
3. Thromboplastin generation test.
4. Thrombin clot time.
5. Antihemophilic globulin (Factor VIII).
6. Plasma thromboplastin antecedent (Factor XI).
7. Recalcification time and nonspecific mixing test for anticoagulants.
8. Proaccelerin (Factor V).
9. Proconvertin (Factor VII).
10. Plasma thromboplastin component (Factor IX).
11. Hageman factor (Factor XII).
12. Stuart factor (Factor X).

The results of the coagulation tests are given in Tables I and II and in the Figure. The clotting times of honey bee hemolymph in each test were at least 2-3 times as long as that of human plasma. The absence of proconvertin from the hemolymph prevented the formation of a clot in the test of one-stage prothrombin time.

Table I. Results of coagulation tests

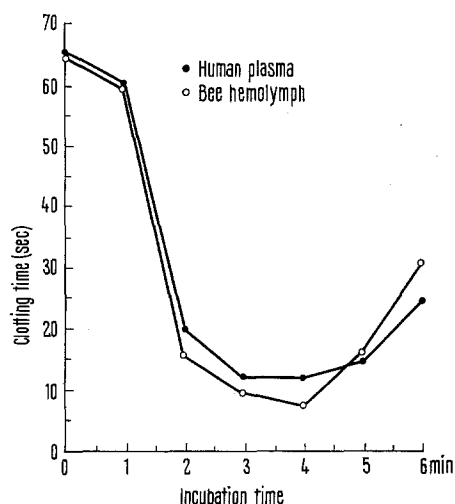
Coagulation test	Coagulation time ^{a, b}	
	Human plasma	Larval hemolymph
One-stage prothrombin time	18.8	> 2 h
Partial thromboplastin time	53.2	365.8
Thrombin clot time	10.8	> 2 h
Antihemophilic globulin	88.3	150.3
Plasma thromboplastin antecedent	92.5	217.0
Proaccelerin	64.0	203.0
Proconvertin	24.2	> 2 h
Plasma thromboplastin component	83.5	285.0
Hageman factor	186.2	355.3
Stuart factor	20.2	372.4

^a Average of 3 determinations. ^b In seconds unless otherwise noted.

Table II. Results of recalcification time and nonspecific mixing test for anticoagulants

Substrate	Clotting time ^a
0.5 ml human plasma	3 min
0.5 ml larval hemolymph	> 2 h
0.25 ml human plasma, 0.25 ml larval hemolymph	4 min

^a Average of 3 determinations.



Results of thromboplastin generation test.

Table II shows that the larval hemolymph lengthened the recalcification time of human plasma. Thus, a circulating anticoagulant and the absence of proconvertin may possibly account for the negative thrombin clot time of bee blood.

The thromboplastin generation time curves of larval hemolymph and human blood (Figure) were similar. Therefore, the hemolymph was capable of generating intrinsic thromboplastin with the aid of human sera and platelets. The concentration of tissue thromboplastin was negligible, but the partial thromboplastin concentration was detectable.

The clotting system for honey bee hemolymph was similar to that of man, except that honey bee hemolymph had no proconvertin and contained lesser amounts of other coagulants; as noted, a circulating anticoagulant probably was present in hemolymph. The coagulants in bee hemolymph might have had a different chemical nature and thus were less suitable substrates, enzymes, or co-factors in the system.

Zusammenfassung. Haemolympe der Honigbiene *Apis mellifica* wurde auf das Vorhandensein von verschiedenen Koagulantien untersucht, die im menschlichen Blut vorkommen. Die Haemolympe gerann nicht, weil sie kein Proconvertin enthielt. Zudem konnte ein zirkulierendes Anticoagulans nachgewiesen werden.

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³ C. GREGOIRE, *Smithson. misc. Collns* 139, 1 (1959).

⁴ J. W. EICHELBERGER, *Laboratory Methods in Blood Coagulation* (Harper and Row, New York 1965).

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Graft Versus Host Reactivity of Embryonic Lymphoid Cells, Activated by Irradiated Thymus and/or Bursa Cells

The function of the thymus and the bursa of *Fabricius* in chicken was the subject of many investigations up to now. Most of the experiments reported¹⁻⁴ were concerned with the loss of certain properties of an immunological nature by chickens thymectomized or bursectomized, either surgically or hormonally.

Our approach^{5, 6} was based on the 'Simonsen phenomenon', i.e. production of lesions on the chorio allantoic membrane of chick embryos by immunologically competent cells. In a previous work⁷ we showed that allogenic bursa cells could cause isologous lymphocytes to give a graft versus host (GVH) reaction on chick embryos. We found the same to be true for thymus cells, which led us to the conclusion that the phenomenon was a result

of the action of a humoral factor, contained in bursa and thymus cells of chicken, that activated the lymphocytes⁸.

In the present experiment we tried to find out whether this was caused merely by the activation of pre-existing competence or else that the thymus and/or the bursa could also affect the embryonic cells by changing them from inactive cells into active ones⁹.

Though it was already stated^{10, 11} that in chickens neither bursa nor thymus cells can give a GVH reaction, the question was raised whether bursa or thymus cells could not be changed during contact with other cells, prior to inoculation on the chorio allantoic membrane, so as to make them capable of producing lesions.